131 Surveillance of *Pseudomonas aeruginosa* infections at the Prague CF centre

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Pseudomonas aeruginosa (PA) causes chronic respiratory infections in 40–70% of adult CF patients. The infection can spread between patients as documented by identification of transmissible strains in some CF centres.

The aim of this study was to set up a surveillance program for PA infections in the Prague CF centre and to map a local epidemiological situation.

In 2004–2012, we collected 865 PA isolates (244 patients, 1–18 samples/patient). Three methods were compared for their suitability to be used for surveillance at the (i) intra- and (ii) inter-patient level: random amplified polymorphic DNA (RAPD), multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE).

We genotyped 350 isolates (196 pts) using RAPD; 225 of them (153 pts) were also examined by MLST and 64 isolates (42 pts) by PFGE. While all methods provided concordant results for strain carriage monitoring at the intrapatient level, RAPD failed to distinguish interpatient isolates whose unrelatedness was apparent from other two typing methods.

The analysis

i. was performed with RAPD in 90 patients: the replacement of one PA strain with another was observed in 19 cases (21%). The MLST-based analysis
ii. revealed great heterogeneity between PA isolates (107 STs in 153 patients; single ST shared by max. 10 patients).

The surveillance protocol composes of MLST for characterization of initial isolates and RAPD for subsequent isolates. If an RAPD pattern of a new isolate differs from the previous one, MLST is carried out to confirm strain replacement. An observed diversity in the PA population is indicative of accurate anti-epidemic measures set at the CF centre.

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132 Anti-*Pseudomonas aeruginosa* IgG ELISA in a CF referral center in Brazil. Preliminary results

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Objectives: The purpose of this study was to standardize and detect IgG antibodies against *P. aeruginosa* in sera of patients from a referral Cystic Fibrosis center in Brazil, using the ELISA technique.

Methods: Sera from 58 patients diagnosed with CF followed at the Hospital of the University of Campinas were evaluated for IgG antibodies against *P. aeruginosa*. Patients were previously classified taking into account the *P. aeruginosa* culture profile of colonization/infection following the criteria of Lee et al. (2003). For the determination of antibodies we are using the ELISA IgG Pseudomonas CF®, Statens Serum Institute (Copenhagen, Denmark). The serology results were compared to the results of microbiological culture, to verify the agreement between them.

Conclusion: Fifty eight patients (age 0.8–26.0; mean 9.3 y), and according to microbiological culture, 6.9% were chronic patients, 22.4% intermittent, 37.9% free of infection, 32.7% never colonized by *P. aeruginosa*. For the first sample analysed positive serology were find for all chronic patients, 61.5% of intermittent, 58.3% of free and 29.4% of never colonized. The high number of patients with positive serology among intermittent may suggest that they are probably infected with *P. aeruginosa*, the same occurring for patients classified by culture as free of infection. An apparent important lack of concordance was observed for patients never colonized with *P. aeruginosa*, where 29.41% (5 patients) had positive serology, which might be due to cross-reactivity, cutt-off, or even the limitations of culture. These are preliminary results and should be further analysed with the follow-up of these patients.

133 Usefulness of real-time PCR in diagnosing initial *Pseudomonas aeruginosa* infection in cystic fibrosis patients

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Objectives: Highly sensitive and specific methods are needed to timely identify *P. aeruginosa* (*PA*). We tested the Real-Time (RT) PCR assay method to identify *PA* growth from respiratory specimens comparing it with microbiological cultures.

Methods: 754 samples from 96 patients categorized in: "chronic infection" (16 pts, mean age of 17.7 ys), "intermittent infection" (29 pts, mean age of 8 ys), or "negative" (51 children, mean age of 6 ys) were processed with RT-PCR assays in addition to conventional microbiological cultures. The DNA samples extracted were analyzed for *PA* by a qualitative real-time PCR assay, BioDetect *P. aeruginosa* (Biodiversity), according to the manufacturer's instructions on the 7500 Real-Time PCR System instrument (Applied Biosystem).

Results: In the intermittent group (191 samples), 18/59 (30.5%) were found to be positive only using the PCR method. In the negative group (404 samples), 13/39 (33%) specimens were *PA* positive only with PCR. No false negative PCR results were detected when conventional cultures were positive, but in total 38 specimens were PCR positive with a negative culture growth. Positive PCR results (with negative cultures) were followed by more intensive clinical and microbiological surveillance. The greater sensitivity of Real-Time PCR to cultures in identifying also a very low bacterial charge allows an earlier diagnosis, as in 7 "negative" subjects, RT PCR detected the *PA* presence before the conventional microbiological test did.

Conclusion: RT-PCR test was more sensitive in identifying *PA* than microbiological cultures. The usefulness of RT-PCR should be also tested in the follow-up of eradication therapy efficacy.

134 Serology and clinical parameters for diagnosis of *Pseudomonas aeruginosa* infection in children with cystic fibrosis

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Pseudomonas aeruginosa (Pa) early pulmonary infection in children with Cystic Fibrosis (CF) is difficult to diagnose. Serology has been used as a useful tool to detect it.

Objectives: Determine clinical factors associated with Pa infection in CF children and their serologic status.

Methods: Cross sectional analysis of clinical parameters (year before serology) and of antibodies against 3 Pa antigens (alkaline protease; elastase; exotoxin A; Mediagnost™, Reutling, Germany) in children with CF. Pa infection was determined based on regular culture (respiratory secretions) on the previous year. Results were compared with culture (reference standard). Positive serology: $\geqslant$1 antigen positive determination.

Results: 40 patients were included (10.5 years [1–21], 60% girls). Pa infection was established: chronic in 8 patients (all were seropositive), intermittent in 17 (88.2% seronegative); 15 non-infected (all seronegative). Serology had 100% specificity, 40% sensitivity (kappa = 0.33), 100% positive predictive value (PPV), 50% negative predictive value (NPV) for Pa infection and accuracy 62.5%. Those with positive serology (n = 10) had significant lower median FEV1 (64%P [32–87] vs 91 [37–128], p = 0.03) and had a tendency for lower BMI z-score (–0.9 [–2.3–0.3] vs –0.2 [–2.8–1.6], p = 0.06). Treatment duration, genotype and pancreatic function were similar.

Conclusions: Pa seropositive patients had lower lung function. Serology was specific for Pa infection and had high PPV, but sensitivity and NPV were lower than in previous studies. Serology may be considered as adjuvant to culture but clinical and serologic follow-up is needed to verify if seroconversion indicates evolution to Pa chronicity.